Docket No.: 532732000101
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:
Yajun GUO et al.

Application No.: 09/915,746 Confirmation No.: 1834

Filed: July 26, 2001 Art Unit: 1642

For: METHOD AND COMPOSITION FOR DIAGNOSIS OF MELANOCYTIC LESIONS Examiner: K. Canella

DECLARATION OF YAJUN GUO PURSUANT TO 37 C.F.R. §1.132

MS Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

I, Yajun Guo, declare as follows:

1. I have a Ph.D. in Immunology from the Second Medical University at Shanghai, P. R. China; and a M.D. from the Third Medical University at Chongqing, P.R. China. I am currently professor at Eppley Institute for Cancer Research of UNMC Cancer Center, Nebraska Medical Center, Omaha, U.S.A.; and professor and president at Shanghai International Joint Cancer Institute and Tumor Immunology & Gene Therapy Center, and professor and deputy director of Shanghai Eastern Institute & Hospital of Hepatobiliary Surgery at Shanghai Second Medical University, P. R. China. I have been co-authored over 120 publications and 8 books.

2. I am one of the joint inventors of the subject matter specifically claimed in the above-referenced patent application U.S. Ser. No. 09/915,746, and I am familiar with the contents thereof.

3. I am also a co-author of the reference (Chen et al., J. Mol. Med. 76: B11 (1998)) cited by the Examiner. I am familiar with the contents thereof.

4. The experiments described by Chen et al. were not repeatable in my laboratory at Shanghai Second Medical University, and the data described in the abstract of Chen et al. were not published in any scientific article after publication of the abstract of Chen et al.

5. Experiments described below were carried out in my laboratory at Shanghai Second Medical University, Shanghai, P. R. China. Data presented in Exhibits A and B demonstrate that the antigen that antibody SM5-1 specifically binds is not a fibronectin.

6. Flow cytometry was performed to demonstrate that antibody SM5-1 does not bind to a cell line expressing fibronectin. HK-2 (ATCC CRL2190) is a human kidney cortex cell line that is known to express fibronectin. QYC is a human primary hepatoma cell line established in my laboratory and has been shown to express the antigen that antibody SM5-1 specifically binds.

7. Anti-fibronectin antibody (Jingmei Biotech, OBT 0082S) is a mouse monoclonal antibody that binds to an epitope common to all human fibronectin proteins. HK-2 cells were incubated with the anti-fibronectin antibody (5 ug/ml) as primary antibody or incubated with the anti-fibronectin antibody (5 ug/ml) in the presence of fibronectin (20 ug/ml) (1918-FN-02M, R&D) on ice for 45 min. Then the cells were washed with PBS for 2 times. The cells were then incubated with FITC-goat anti-mouse antibody as secondary antibody on ice for 45 min. After being washed with PBS for two times and resuspended in PBS, the cells were run through FACS. As shown in Exhibit A, the anti-fibronectin antibody bound to the HK-2 cells (Figure 1) and the binding was blocked by fibronectin (Figure 2).

8. QYC cells were incubated with antibody SM5-1 (5 ug/ml) as primary antibody or with the antibody SM5-1 (5 ug/ml) in the presence of fibronectin (20 ug/ml) on ice for 45 min. Then the cells were washed with PBS for 2 times. The cells were then incubated with FITC-goat anti-mouse antibody as secondary antibody on ice for 45 min. After being washed with PBS for two times and resuspended in PBS, the cells were run through FACS. As shown in Exhibit A antibody SM5-1 bound to the QYC cells (Figure 3) and the binding was not blocked by fibronectin (Figure 4).

9. The flow cytometry data presented in Exhibit A demonstrate that fibronectin does not block binding of SM5-1 to its antigen, but fibronectin blocks an anti-fibronectin antibody to fibronectin. These data indicate that SM5-1 does not bind to fibronectin.

10. ^{35}S-metabolic labeling followed by immunoprecipitation was also performed to show that antibody SM5-1 does not bind to fibronectin. ^{35}S-methionine was used to label proteins in QYC and HK-2 cells. After incubation in a cell culture medium with ^{35}S-methionine for 10 hours, cells were lysed and cell lysate was immunoprecipitated with an antibody (50 ug/ml) described below in the absence or presence of fibronectin (50 ug/ml). Immunoprecipitates were loaded on a SDS-PAGE, and the SDS-PAGE was dried and exposed to a film. The result is shown in Exhibit B. Molecular weight markers are shown in Lane 1. HK-2 cell lysate immunoprecipitated with the anti-fibronectin antibody is shown in Lane 2. Two bands with molecular weight of about 250 kDa and about 200 kDa were observed in Lane 2. HK-2 cell lysate immunoprecipitated with the anti-fibronectin antibody in the presence of fibronectin is shown in Lane 3. No band was observed in Lane 3. QYC cell lysate immunoprecipitated with the anti-fibronectin antibody is shown in Lane 4. No band was observed in Lane 4. HK-2 cell lysate immunoprecipitated with a control antibody (Jingmei Biotech 50080-1) is shown in Lane 5. No band was observed in Lane 5. QYC cell lysate immunoprecipitated with antibody SM5-1 in the presence of fibronectin is shown in Lane 6. Two bands with molecular weight of about 230 kDa and about 180 kDa were observed in Lane 6. HK-2 cell lysate immunoprecipitated with antibody SM5-1 is shown in Lane 7. No band was observed in Lane 7. QYC cell lysate immunoprecipitated with a control antibody (Jingmei Biotech 50080-1) is shown in Lane 8. No band was observed in Lane 8. QYC cell lysate immunoprecipitated with antibody SM5-1 is shown in Lane 9. Two bands with molecular weight of about 230 kDa and about 180 kDa were observed in Lane 9.

11. Data shown in Exhibit B demonstrate that an anti-fibronectin antibody binds to proteins with molecular weight of about 250 kDa and about 200 kDa in HK-2 cells, but does not bind to any proteins in QYC cells. In contrast, antibody SM5-1 binds to proteins with molecular weight of about 230 kDa and about 180 kDa in QYC cells, and this binding cannot be competed by fibronectin. Antibody SM5-1 does not bind to any proteins in HK-2 cells. These experiments indicate that the antigen that antibody SM5-1 specifically binds is not fibronectin or fibronectin variants.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application, any patent issuing thereon, or any patent to which this verified statement is directed.

Dec. 29. 06
Date

Yajun Guo

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants:

Jing Ma

Serial No.: 09/451,353

Filed: December 1, 1999

For: METHOD AND COMPOSITION FOR DIAGNOSIS OF MELANOCYTIC LESIONS

Group Art Unit: 1642

Examiner: Canella, K.

DECLARATION OF JING MA

1. I am the inventor of claims 1-18, as amended, of the patent application serial number 09/451,353, filed December 1, 1999. I have reviewed the Office Action for that application mailed May 9, 2000.

2. A hybridoma secreting SM5-1 antibody was deposited with America Type Culture Collection 10801 University Boulevard, Manasses, VA, 20110-2209 USA, on October 20, 1998 under ATCC Accession No. HB-12588. All restrictions upon public access to the deposit will be irrevocably removed upon grant of a patent on this application. The deposit will be replaced if viable samples cannot be dispensed by the depository.

I declare that all statements made herein are based on my own personal knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 10001 of Title 18 of the

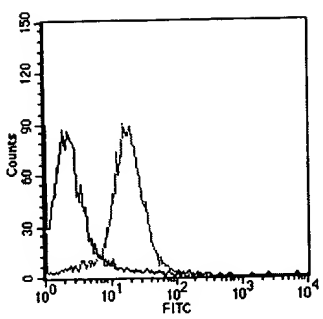
United States Code, and that such willful false statements may jeopardize the validity of the application or any patents issued thereon.

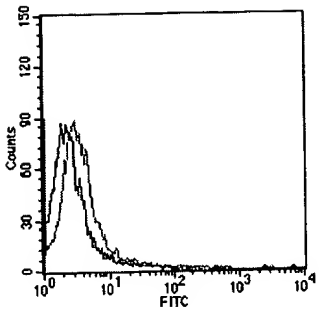
Dated: 11/12/00

Jing Ma

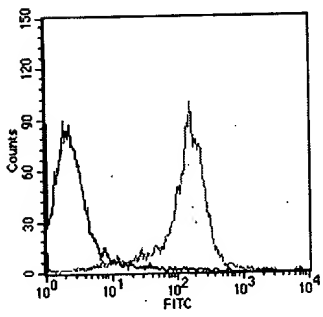
Jing Ma

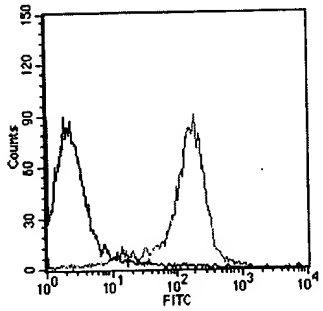
Exhibit A





Left: Figure 1
Right: Figure 2





Left: Figure 3
Right: Figure 4

Exhibit B